diluted with equal volume of chloroform and the spectra scanned between 400-700 nm.

5.2. RESULTS

5.2.1. Trials with reducing systems:

5.2.1.1. Results of trials with the naturally occurring xanthine oxidase of milk by providing substrates xanthine and acetaldehyde in aseptically collected milk are tabulated in Table 3. It was noticed that though there was no formation of ether extractable bilirubin, there was always a decrease in the concentration of biliverdin present in the experimental samples, while, the sample in which the enzyme activity was destroyed by maintaining milk at 80°C for 30 sec contained almost the same quantity of biliverdin as aseptic milk which was used as control. Further, in the experimental samples where the xanthine oxidase system was activated, the casein was coloured yellow. In a few trials, however, a minor peak at 420 nm was noticed in the enzyme activated samples (Figs. 8 & 9).

5.2.1.2. Almost similar observations were made when chemical reducing agents like ascorbic acid (Fig. 10) and cysteine were added to aseptic milk. Results of these trials are tabulated in Table 4.

5.2.1.3. However, in trials carried out by providing aldehyde dehydrogenase enzyme system to aseptic milk, practically no change
FIG. 8—ABSORPTION SPECTRUM OF THE 420 nm PIGMENT FORMED IN ASEPTIC MILK
Optical density

FIG. 9-ABSORPTION SPECTRUM OF THE 420 nm PIGMENT FORMED IN ASEPTIC MILK

- Control sample
- Acetaldehyde added sample

FIG. 9 - ABSORPTION SPECTRUM OF THE 420 nm PIGMENT FORMED IN ASEPTIC MILK
FIG. 10 - ABSORPTION SPECTRUM OF THE 420 nm PIGMENT FORMED IN ASEPTIC MILK
was noticed between the experimental and control samples in the concentration of biliverdin present at the end of the incubation period (Table 3).

5.2.2. Trials with reducing systems combined with proteolytic systems:

5.2.2.1. Results obtained on trials done by inducing a reducing system combined with a proteolytic system in aseptic milk, by using the native xanthine oxidase of milk or chemical reductants as reducing systems combined with papain or rennin as proteolytic system simultaneously are tabulated in Tables 5 & 6. It was noticed that there was almost a 100% conversion of biliverdin to bilirubin in xanthine oxidase activated system combined with the proteolytic system in about 15 h time while in chemical reducing system combined with the proteolytic system there was about 80% conversion of biliverdin to bilirubin (Figs. 11 & 12).

5.2.2.2. It is clear from the results presented above that the reduction of biliverdin to bilirubin in milk appears to be a non-specific reduction and that a release mechanism is needed to set free the bilirubin formed from the protein matrix which holds the pigment. The bilirubin formed by induced reducing systems alone, is not extractable. On the other hand, when microorganisms bring about the conversion of biliverdin to bilirubin in milk, they seem to bring about the dual change of reduction and release, since bilirubin formed in such cases is easily extractable in ether.
FIG. 11 - ABSORPTION SPECTRUM OF BILIRUBIN FORMED IN ASEPTIC MILK BY THE COMBINED ACTION OF REDUCTION AND PROTEOLYSIS
Optical density

Control sample
Aseptic milk + Ascorbic acid + Papain
Aseptic milk + Cysteine + Papain

FIG. 12—ABSORPTION SPECTRUM OF BILIRUBIN FORMED IN ASEPTIC-MILK BY THE COMBINED ACTION OF REDUCTION AND PROTEOLYSIS
5.2.2.3. This is further substantiated by the observation made earlier under 4.2.6.4, wherein, when the conversion of the green pigment to the yellow was brought about by the microorganisms in milk, inspite of a rapid fall in the concentration of the green pigment by about 9 h of incubation, the formation of the yellow pigment was very gradual with more or less a constant increase till 24 h suggesting a gradual release of the preformed yellow pigment by some agency.